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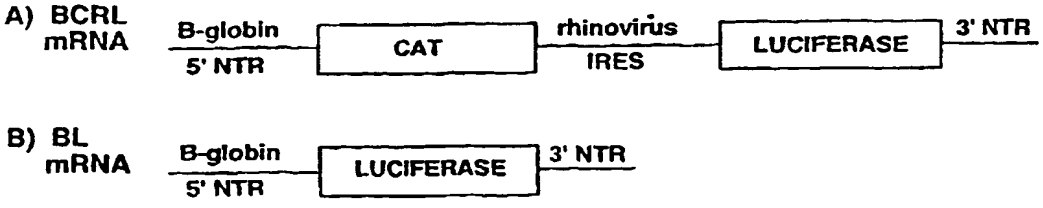

(57) Abstract

Method for screening for an antiviral agent, by determining whether a potential agent interacts with a virus or cellular component which allows or prevents preferential translation of a virus RNA compared to a host RNA under virus infection conditions; and determining whether any interaction of the agent with the component reduces the level of translation of an RNA of the virus.

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Claims

1. Method for screening for an antiviral agent, comprising the steps of:

determining whether a potential said agent interacts with a virus or cellular component which allows or prevents preferential translation of a virus RNA compared to a host RNA under virus infection conditions; and

determining whether any interaction of said agent with said component reduces the level of translation of a RNA of said virus.

2. The method of claim 1, wherein said component is a protein or a nucleic acid.

3. The method of claim 2, wherein said component is virus encoded.

4. The method of claim 2, wherein said component is host cell encoded.

5. The method of claim 1, wherein said component is a macromolecule selected from the group consisting of:

an RNA sequence domain, a DNA sequence domain, an initiation factor, and elongation factor, a termination factor, a transcription factor, a ribosomal protein, a glycosylase, a deglycosylase, a prenylating and deprenylating enzyme, a transferase, a polymerase, a synthetase, an ADP ribosylating enzyme, an ADP ribosylase, a kinase, a lipase, a myristylating or demyristylating enzyme, a phosphorylase, a protease, a rRNA, a tRNA, a ribonuclease, and a deoxyribonuclease.

6. The method of claim 5, wherein said sequence domain is translationally linked to RNA encoding a reporter polypeptide, and said second determining step

includes determining whether said agent alters the level of translation of said reporter polypeptide.

7. The method of claim 1, wherein said component is a protein or a polypeptide, and said determining steps include providing said component in a translation mixture with RNA encoding a reporter polypeptide, and determining whether said agent alters expression of said reporter polypeptide in said mix.

8. The method of claim 5, wherein said RNA sequence domain is selected from the group consisting of:

an IRES sequence, a 5'-untranslated region, a 3'-untranslated region, and an upstream open-reading frame sequence.

9. The method of claim 1, wherein said component is selected from the group consisting of:

a double-stranded RNA-activated protein kinase, and an inhibitor of a double-stranded RNA-activated protein kinase.

10. The method of claim 9, wherein said component is selected from the group consisting of:

p68 kinase, VAI RNA, TAR of HIV genome, EBER-1 RNA, and p58.

11. The method of claim 1, comprising the steps of:

forming a protein translation mixture which includes (i) a viral mRNA construct, said mRNA construct comprising (a) an internal ribosome entry site (IRES) region and downstream of said IRES region, a first reporter protein coding region, (ii) ribosomes, and (iii) an agent to be tested,

incubating the components of the translation mixture under conditions effective to produce from the

first reporter protein coding region a reporter protein, and examining the mixture for the presence of reporter protein produced by such translation mixture, wherein said agent is a useful antivirus agent if the reporter protein produced in the presence of the test agent is less than an amount of reporter protein produced in the absence of said test agent.

12. The method of claim 11, wherein the IRES region is derived from a picornavirus IRES region sequence.

13. The method of claim 12, wherein the IRES sequence is selected from the group consisting of an enterovirus, rhinovirus, cardiovirus, and aphthovirus IRES sequence.

14. The method of claim 11, wherein said IRES region is selected from the group consisting of an hepatitis A virus IRES sequence, an hepatitis B virus sequence and an hepatitis C virus IRES sequence.

15. The method of claim 11, wherein the protein translation mixture is a cell-free extract.

16. The method of claim 11, wherein the 5'-end of the viral mRNA construct includes a eukaryotic mRNA 5'-terminal cap and untranslated region (UTR) and downstream of said cap and UTR region, a second reporter protein.

17. The method of claim 16, wherein the translation mixture is contained in a cell.

18. The method of claim 1, comprising the steps of:

forming a binding mixture comprising a cellular or viral translation initiation protein, an IRES element

ribonucleotide sequence, and an agent to be tested,

incubating the components of the binding mixture under conditions effective to bind the initiation protein to the IRES element, and

examining the mixture for the presence of binding of the initiation protein to the IRES element;

wherein said agent is a useful anti virus agent if the extent of binding of the initiation protein to the IRES element is less than that observed in the absence of said agent.

19. The method of claim 18, wherein the cellular or viral translation initiation protein is selected from the group consisting of p52 and p57.

20. The method of claim 18, wherein the IRES element ribonucleotide sequence is derived from a picornavirus IRES region sequence.

21. The method of claim 20, wherein the IRES sequence is selected from the group consisting of an enterovirus, rhinovirus, cardiovirus, and aphthovirus IRES sequence.

22. The method of claim 18, wherein said IRES region is selected from the group consisting of an hepatitis A virus IRES sequence, an hepatitis B virus sequence and an hepatitis C virus IRES sequence.

23. The method of claim 18, wherein the cellular or viral translation initiation protein is bound to a solid support, the IRES element is labeled with a reporter, and said examining includes measuring the amount of reporter bound to the solid support.

24. The method of claim 18, wherein the IRES element RNA is bound to a solid support, the cellular or

viral translation initiation protein is labeled with a reporter, and said examining includes measuring the amount of reporter bound to the solid support.

25. The method of claim 24, wherein a terminal region of said IRES element is bound to a complementary DNA sequence, and said DNA sequence is linked to the solid support.

26. The method of claim 18, further including the step, after said incubating step, of adding to the incubation mixture an RNAase capable of cleaving free RNA but not protein bound RNA, and wherein said binding of the initiation protein to the IRES element is detected by the presence in the mixture of uncleaved IRES element RNA.

27. The method of claim 18, wherein said examining includes subjecting said mixture to a gel-shift electrophoresis assay.

28. The method of 18, wherein said incubating is carried out in solution phase, and said examining includes passing the mixture through a filter which retains said IRES element only when the element is bound to the cellular or viral translation initiation protein.

29. A method of treating a subject infected with a virus, comprising the steps of:

administering, in a therapeutically effective dose, an agent which interacts with a viral or cellular component which allows or prevents preferential translation of a virus RNA compared to a host RNA under virus infection conditions.

30. The method of claim 29, wherein said virus is a single-strand RNA virus having an IRES region, and said agent blocks the <u>in vitro</u> binding of a cellular or

viral translation initiation protein to an IRES region.

31. The method of claim 29, wherein said virus is hepatitis B virus, and said agent blocks the in vitro binding of a cellular or viral translation initiation protein to an IRES region.

32. The method of claim 30, for treatment of poliovirus infection, wherein the agent administered is able to block in vitro binding of cellular or viral translation initiation protein p52 to an poliovirus-derived IRES region.

33. The method of claim 29, wherein said method comprises inhibiting viral replication in a host eukaryotic cell, where the virus produces a viral inhibitor which interferes with the activation of the host-cell interferon-induced, double-stranded RNA-activated protein kinase, comprising the steps of:

administering to the cells, an agent able to block the effect of the viral inhibitor in interfering with the activation of the protein kinase.

34. The method of claim 33, wherein the virus produces a RNA viral inhibitor able to block binding of double-stranded RNA to the protein kinase, and the agent administered is able to block the binding of the viral inhibitor to the protein kinase.

35. The method of claim 33, wherein the agent is selected by the steps of:

incubating a mixture containing the protein kinase, viral inhibitor, and agent to be selected under conditions effective to bind the protein kinase to the viral inhibitor, in the absence of the agent, and

examining the mixture for the presence of binding of the protein kinase to the viral inhibitor, to

determine whether the presence of the agent has inhibited binding of the protein kinase to the viral inhibitor.

36. The method of claim 33, wherein the agent is selected by the steps of:

incubating a mixture containing the protein kinase, the viral inhibitor, and agent to be tested, under conditions effective to activate the protein kinase in the absence of the viral inhibitor, and

examining the mixture for the presence of protein kinase activity.

37. The method of claim 29, wherein the virus is adenovirus, and the viral inhibitor is a VAI RNA molecule.

38. The method of claim 29, wherein the virus is human immunodeficiency virus (HIV), and the viral inhibitor is a TAR region of the HIV genome.

39. The method of claim 29, wherein the virus is an Epstein-Barr virus, and the viral inhibitor is an EBER-1 RNA.

40. The method of claim 29, wherein the viral inhibitor is effective to activate a host-cell p58 protein which is able, in activated form, to block the activation or activity of the protein kinase, and said agent blocks the interaction of the viral inhibitor with the p58 protein.

41. The method of claim 40, wherein the agent is selected by the steps of:

incubating a mixture containing the protein kinase, the p58 protein, in non-activated form, and the agent, under conditions effective to activate the protein kinase, when the p58 protein is absent, and

examining the mixture for the presence of protein kinase activity.

42. The method of claim 32, wherein said component is a viral protein selected from the group consisting of:

vaccinia E3L protein, a rotavirus protein, a reovirus s3 protein which binds to activating double stranded RNA, a poliovirus 2A protein which degrades p68.

43. The method of claim 1, wherein said agent is effective to inhibit viral replication in a host eukaryotic cell, where the virus produces an inhibitor which interferes with the activation or activity of the host-cell interferon-induced, double-stranded RNA-activated protein kinase, comprising

incubating a mixture containing the protein kinase, the inhibitor, and the agent to be tested under conditions effective to cause inhibitor interference with the activation or activity of the protein kinase, and

examining the mixture for such interference.

44. The method of claim 1, wherein said agent is effective to inhibit viral replication in a host eukaryotic cell, where the host cell produces an inhibitor which interferes with the activation of the host-cell interferon-induced, double-stranded RNA-activated protein kinase, comprising

incubating a mixture containing the protein kinase, the inhibitor, and the agent to be tested under conditions effective to cause inhibitor interference with the activation of the protein kinase, and

examining the mixture for such interference.

45. The method of claim 43, for use in screening an agent effective to inhibit replication in a host cell of a virus which produces an inhibitor able to

bind to the protein kinase, to interfere with the activation of the protein kinase by double-stranded RNA, wherein said incubating includes incubating the protein kinase, viral inhibitor, and agent under conditions effective to bind the inhibitor to the protein kinase, and said examining includes examining the protein kinase for bound inhibitor.

46. The method of claim 45, wherein said incubating is carried out in solution phase, and said examining includes passing the protein kinase, viral inhibitor, and test agent through a filter which retains the inhibitor only when the inhibitor is bound to the protein kinase.

47. The method of claim 45, wherein the protein kinase is bound to a solid support, the inhibitor is labeled with a reporter, and said examining includes measuring the amount of reporter bound to the solid support.

48. The method of claim 43, wherein said incubating is carried out under conditions in which the protein kinase is autophosphorylated, in the absence of binding to the viral inhibitor, and said examining includes determining the extent of phosphorylation of the p68 kinase.

49. The method of claim 43, for use in screening agents effective in blocking viral replication of a virus which produces an inhibitor effective to activate a p58 host-cell protein which in activated form is effective to block activity or activation of the protein kinase, wherein the mixture formed includes the p58 host-cell protein, said incubating is carried out under conditions in which the protein kinase is activated in the absence of p58, and said examining includes

examining the mixture for inhibition of protein kinase activity.

50. The method of claim 43, wherein the protein kinase and inhibitor are expressed in a yeast cell which is constructed to increase the expression of a reporter protein in the presence of activated protein kinase, and said examining includes examining the yeast cells for increased expression of the reporter protein.

51. The method of claim 50, wherein the reporter protein is fused GCN4/β-gal protein.

52. A yeast cell for use in screening agents effective to inhibit viral replication in a host eukaryotic cell, where the virus produces a viral inhibitor which interferes with the activation of the host-cell interferon-induced, double-stranded RNA-activated protein kinase, comprising:

(a) an expressed gene encoding a mammalian interferon-induced, double-stranded RNA-activated protein kinase,

(b) a reporter gene whose expression in increased by activation of the protein kinase, and

(c) a viral gene for producing a viral inhibitor able to block activation of the protein kinase.

53. The yeast cell of claim 52, wherein the reporter gene is a fused GCN4/β-gal gene.

54. The method of claim 40 wherein said agent blocks interaction of p56 and p68.

55. A yeast cell for use in screening agents effective to inhibit viral replication in a host eukaryotic cell, where the virus activates or induces a cellular protein to interfere with the activation of the

host-cell interferon-induced, double-stranded RNA-activated protein kinase, comprising:

(a) an expressed gene encoding a mammalian interferon-induced, double-stranded RNA-activated protein kinase,

(b) a reporter gene whose expression in increased by activation of the protein kinase, and

(c) a gene encoding a protein which blocks activation of a cellular protein.

56. A method for inhibiting the activity of a viral nucleic acid product which inhibits the function of a cellular component which regulates translation comprising the step of administering a nucleic acid fragment complementary to at least a portion of said viral nucleic acid product.

57. The method of claim 56 wherein said cellular component prevents the translation of viral RNA.

58. The method of claim 56 wherein said cellular component is p68.

59. The method of claim 56 wherein said nucleic acid fragment is selected from the group consisting of DNA, RNA, modified DNA and modified RNA.

60. The method of claim 56 wherein said viral nucleic acid product is VAI.

61. The method of claim 56 wherein said nucleic acid fragment complementary to at least a portion of said viral nucleic acid product is ava 1.

62. The method of claim 56 wherein said nucleic acid fragment complementary to at least a portion of said viral nucleic acid product is either ava 9 or ava 15.

63. A composition which inhibits the activity of a viral nucleic acid product wherein said viral nucleic acid product inhibits the function of a cellular component which regulates translation, comprising a composition that is complementary to at least a portion of said viral nucleic acid fragment.

64. The composition of claim 63 wherein said cellular component prevents the translation of viral RNA.

65. The composition of claim 63 wherein said cellular component is p68.

66. The composition of claim 63 wherein said composition is selected from the group consisting of DNA, RNA, modified DNA and modified RNA.

67. The composition of claim 63 wherein said composition is ava 1.

68. The composition of claim 63 wherein said composition is ava 9 or ava 15.

69. A method of inhibiting translation of a nucleic acid containing an IRES wherein said nucleic acid is obtained from a virus, comprising the step of administering to an organism a nucleic acid fragment complementary to at least a portion of said IRES.

70. The method of claim 69 wherein said virus is selected from the group consisting of picornaviruses.

71. The method of claim 69 wherein said virus is selected from the group consisting of rhinovirus, enterovirus, cardiovirus and aphthovirus IRES.

72. The method of claim 69 wherein said virus is selected from the group consisting of hepatitis A, and hepatitis C IRES.

73. The method of claim 71 wherein said rhinovirus is rhinovirus 14.

74. The method of claim 69 wherein said nucleic acid fragment complementary to at least a portion of said IRES is an oligonucleotide comprising a purine tract of about 4 to 12 nucleotides.

75. The method of claim 69 wherein said nucleic acid fragment complementary to at least a portion of said IRES is an oligonucleotide comprising a purine tract of about 5 to 9 nucleotides.

76. The method of claim 74 or 75 wherein said oligonucleotide further comprises a CAT nucleotide triplet located 5' of said purine tract.

77. A method of inhibiting translation of a nucleic acid transcript containing an IRES wherein said transcript is obtained from a hepatitis B virus comprising the step of administering to an organism a nucleic acid fragment complementary to at least a portion of said IRES.

78. A composition comprising a nucleic acid fragment complementary to at least a portion of a viral IRES.

79. The composition of claim 78 wherein said nucleic acid fragment complementary to at least a portion of said IRES is an oligonucleotide comprising a purine tract of 4 to 12 nucleotides.

80. The composition of claim 78 wherein said

nucleic acid fragment complementary to at least a portion of said IRES is an oligonucleotide comprising a purine tract of 5 to 9 nucleotides.

81. The composition of claim 78 wherein said nucleic acid fragment is complementary to a portion of said IRES which contains a Y_nX_mAUG sequence.

82. The composition of claim 78 wherein said nucleic acid fragment is complementary to at least a portion of nucleotides from about 518-551 of a rhinovirus 14 IRES.